Hydrophobicity variation in meat proteins upon thermal treatment

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SUMMARY: A fluorescent probe method using 8-anilino-1-naphtalene sulfonic acid (ANS) and retinol (RET) has been used to study the effects of heat treatments on the meat proteins hydrophobicity. The number of ANS binding sites per unit protein increased from 0.75 for unheated samples to 2.12 for meat proteins heated at 100°C for 30 min. The number of RET binding sites increased from 0.13 to 0.46 with the same heat treatment. This is discussed in terms of % increase in aromatic and aliphatic hydrophobicity of meat proteins.

INTRODUCTION: Heat treatment is an integral part of comminuted meat manufacture. However, this treatment induces protein denaturation and modifications of their functional properties (LI-CHAN et al., 1984). Many papers have been published to emphasize the importance of hydrophobicity in protein functionality.

Fluorescence probes are often used for evaluation of the protein hydrophobicity because of the simplicity and rapidity of the method (SKLAR et al., 1977). In order to asses the aromatic hydrophobicity (ARH), 8-anilino-1-naphtelene sulfonic acid (ANS) seems to be useful since it is composed of aromatic rings (CLARKE and NAKAI, 1972). The utility of retinol as a fluorescent probe for membrane lipids has already been demostrated (RADDA and SMITH, 1970). For determining the aliphatic hydrophobicity (ALH) all-trans-retinol (RET) can be used because it is composed by an aliphatic hydrocarbon chain.

The objetive of the present work was to study the effects of heat treatments on the aromatic and aliphatic hydrophobicity of meat proteins using ANS and RET as fluorescent probes since the quantitation of this parameter can be an essential step for accurate prediction of meat proteins functionality.

MATERIALS and METHODS

Preparation of meat extracts: The muscle tissue used for the preparation of the meat extracts was obtained from the *Muscle rectus femoris* of five different bovine animals.

Salt-extracts of beef samples were prepared in 0.01M sodium phosphate buffer pH 7 containing 0.6M NaCl and 1mM $MgCl_2$, as previously described LI-CHAN et al. (1984).

Extracts were analyzed for protein content by the method of LOWRY et al. (1951).

Heat treatments: Three replicates of each extract in pH 7 buffer were placed in a boiling water bath and heated with constant stirring to attain internal temperatures of 40°C to 100°C and held at the appropiate temperature for 30 min. The solutions were then rapidly cooled in an ice water bath, then homogenised in a Sorvall Omni-mixer homogeneizer (Du Pont Co., Newtown, Conn., USA) at approximately 369 g for 15 sec.

Fluorescent probe method: Determination of apparent meat proteins hydrophobicity was carried out using fluorescent probes : 8-anilino-1-naphtalene sulphonique acid (ANS) and all-trans-retinol (RET) (Sigma Chemical Co., St. Louis, MO, USA).

Each protein sample was diluted with phosphate buffer pH 7 to obtain a protein concentration of 14.5 µg/ml. Solution of ANS 10^{-3} M in phosphate buffer pH 7 and solution of RET 10^{-4} M in ethanol were serially diluted to obtain ANS concentration ranging from 1µg/ml to 100µg/ml and RET concentration ranging from 0.01 µg/ml to 30µg/ml. 500µl of meat proteins solution was added to 2.5 ml of diluted ANS and RET.

The fluorescence intensity (FI) of the ligand-protein conjugates was measured on a Hitachi F-2000 spectrophotometer. ANS-protein conjugates were excited at 370 nm and FI was measured at 450 nm. RET-protein conjugates were excited at 330 nm and FI was measured at 470 nm.

The data analysis was essentially that of COGAN et al. (1976) outlined herein. From mass law considerations,

$$P_0\alpha = -K_d/n + R_0\alpha(1-\alpha) \qquad (1)$$

where P_0 and R_0 are respectively the total protein concentration and the total ligand concentration; K_d, the apparent concentration constant; n, the number of binding sites per unit protein; α, the fraction of free binding sites, is equal to:

$$\alpha = \frac{FI_{max} - FI}{FI_{max}}$$

where FI_{max} is the fluorescence intensity upon saturation of all the protein.

Plotting $P_0\alpha$ *vs* $R_0\alpha(1-\alpha)$ gives a straight line with a slope equal to $1/n$ and intercept of $-K_d/n$.

RESULTS and DISCUSSION: The enhacement of fluorescence of small fluorescent molecules upon binding to proteins has been used here to elucidate the question of the meat proteins hydrophobicity.

The fluorescence intensity values of ligand-protein conjugates when plotted according to Eqn (1) were shown in Fig. 1 and Fig. 2. These Cogan plots for the binding of ANS and RET to meat proteins are linear and give straight lines with a correlation

Figure 1. Cogan plot, for titration of meat proteins with ANS

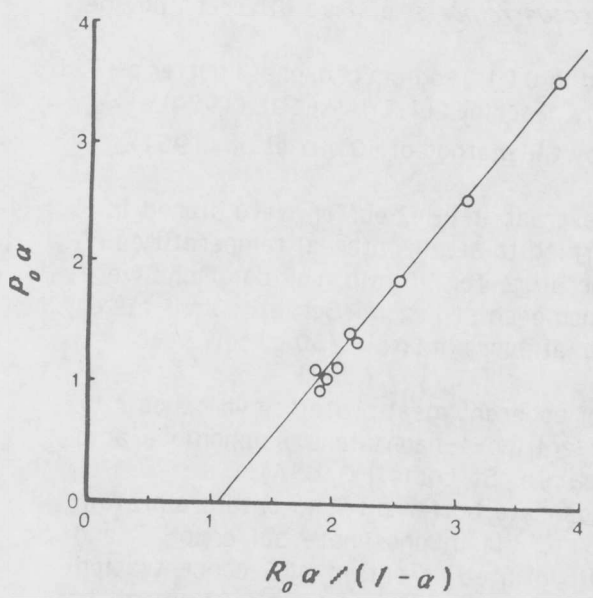
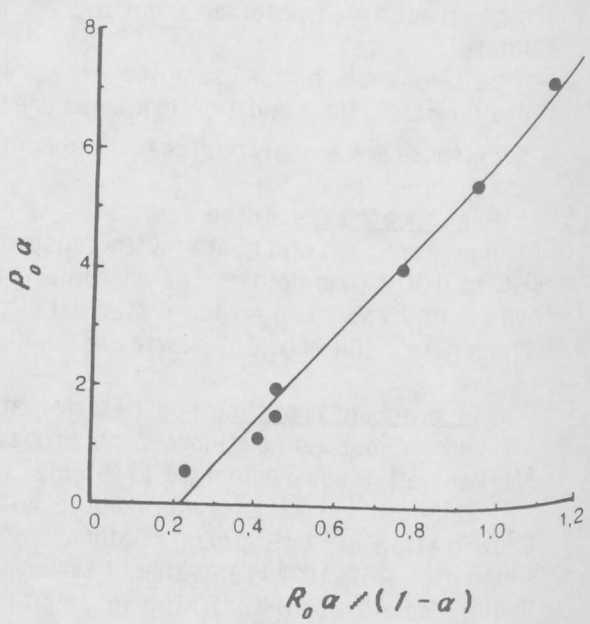


Figure 2. Cogan plot, for titration of meat proteins with RET



coefficient of 0.998 and 0.994 respectively. From the slopes of these straight lines, the number of ANS (N_{ANS}) and RET (N_{RET}) binding sites per unit protein can be calculated.

Table 1 shows the variation of number of ANS and RET binding sites per unit protein as a function of the heat treatments. N_{ANS} increased with heating from 0.75 for unheated samples to 2.12 for meat proteins heated at 100°C for 30 min. N_{RET} increased from 0.13 to 0.46 with the same heat treatment. This increased in number of accesible sites could be explained as result of denaturation proceeds because the hydrophobicity residues buried in the interior are exposed at the molecular surface of proteins.

Table 2 shows the increased in hydrophobicity as a consequence of heating. When heat treatment of 50°C for 30 min was applied, there was a 25% increase of aromatic and aliphatic hydrophobicity. More rigorous treatment was accompanied by a disagrement of hydrophobicity changes. Heating to 60-100°C for 30 min, the % increase of ARH fells within the 36.00-182.66% range, while % increase of ALH fells within the 53.84-253.84% range. Therefore, the hydrophobicity of aliphatic aminoacids was higher than that of aromatic aminoacids, when the heat treatments above 60°C for 30 min were applied.

Linear regression analysis showed that the heat treatment was significantly correlated with the % increase of aromatic ($r = 0.970$ $n = 7$ $P < 0.005$) and aliphatic ($r = 0.985$ $n = 7$ $P < 0.005$) hydrophobicity of meat proteins. The following regresion equations were obtained:

%increase of aromatic hydrophobicity = - 119.442 + 2.789 Heat treatment
%increase of aliphatic hydrophobicity = - 183.247 + 4.313 Heat treatment

The results allowed to deduce that the analysis of the variation of number of ANS or RET binding sites per unit protein interpreted as aromatic and aliphatic hydrophobicity was able to clearly discriminate between samples of meat proteins receiving heat treatments with 10°C intervals.

Table 1. Variation of number of ANS and RET binding sites per unit protein as a function of the heat treatments.

Heat treatment	N_{ANS}	N_{RET}
None	0.75	0.13
40°C - 30 min	0.81	0.15
50°C - 30 min	0.94	0.16
60°C - 30 min	1.02	0.20
70°C - 30 min	1.20	0.27
80°C - 30 min	1.45	0.34
90°C - 30 min	1.69	0.41
100°C - 30 min	2.12	0.46

Table 2. Effect of heating on meat proteins hydrophobicity

Heat treatment	Increase in aromatic hydrophobicity (% ARH)	Increase in aliphatic hydrophobicity (% ALH)
None	-	-
40°C - 30 min	8.00	15.38
50°C - 30 min	25.33	23.07
60°C - 30 min	36.00	53.84
70°C - 30 min	60.00	107.69
80°C - 30 min	93.33	161.53
90°C - 30 min	125.11	215.38
100°C - 30 min	182.66	253.84

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